

## **RESPONSE TO OFFICE ACTION**

### **A. Status of the Claims**

Claims 1, 2, 4-16, and 18-30 were pending at the time of the Action. Claims 15, 16, and 18-29 were withdrawn. Claims 1, 5, 10, 11, and 13 are amended herein. Support for these amendments can be found at Example 5, paragraphs 0041, 0074 and 0081 of the specification, and claims as originally filed. New claim 31 is added herein. Support for this new claim can be found at Example 8, paragraph 0100 of the specification. Claim 9 is cancelled without prejudice in view of the amendment of claim 1. Upon entry of the amendments, claims 1, 2, 4-8, 10-16, and 18-31 will be pending.

### **B. Claim Objections**

The Action objected to claim 5. According to the Action, claim 5 is improper due to the recitation of “about 1-14 days.” Applicant has amended claim 5 to recite “a period of time between about 7 days and about 14 days” so that the claim is in proper dependent form.

In view of the foregoing, withdrawal of the objection is respectfully requested.

### **C. Rejection of Claims Under 35 U.S.C. §112, First Paragraph - Enablement**

The Action rejects claims 1-2, 4-14, and 30 as lacking enablement. In particular, the Action asserts that the specification does not reasonably provide enablement for all types of explants and all transformation methods. Applicants respectfully traverse.

For the reasons discussed in Applicant’s previous Response submitted on September 27, 2007, the instant application provides enabling description for the claims as previously presented. However, in order to advance the prosecution, Applicants have amended claim 1 to

recite, “inserting a nucleic acid comprising a selectable marker gene into a transformable maize tissue by inoculating the transformable maize tissue with *Agrobacteria*” and “the transformable maize tissue comprises an immature maize embryo.” Accordingly, amended claim 1 is directed to the use of immature embryo as a source of explant and the use of *Agrobacteria* as a method of transformation. The specification fully enables this explant and transformation method. For instance, Example 4, paragraph 0074 of the specification teaches that:

Immature embryos are isolated from surface sterilized ears and directly dropped into the prepared *Agrobacterium* cell suspension...After *Agrobacterium* cell suspension is removed using a fine tipped sterile transfer pipette, the immature embryos are transferred onto the co-culture medium (Table 1).

Furthermore, Example 5, paragraph 0081 of the specification describes the following:

Almost all the embryos inoculated and co-cultured with *Agrobacterium* for one day showed excellent GFP gene transient expression, which was demonstrated by a great number of single-celled GFP-positive spots on each embryo.

Accordingly, the specification clearly enables the explant type and transformation method for producing the transgenic maize plant embodied by the claims, and thus enables one of skill in the art.

Additionally, the Action at page 3 states that “the specification...being enabling for *Agrobacterium*-mediated transformation for maize using immature embryo,” and therefore the Action acknowledges that the specification enables the claimed elements discussed above. The rejection is therefore believed moot and removal thereof is respectfully requested.

#### **D. Rejection Under 35 U.S.C. §103**

The Action rejects claims 1-2, 4-14, and 30 as obvious over Frame *et al.*, *Plant Physiology*, 2002, vol. 129, pages 13-22 (“Frame”) in view of Zhao *et al.*, *Molecular Breeding*,

vol. 8, pages 323-222 (“Zhao”) and further in view of U.S. Patent No. 6,329,571, issued to Hiei *et al.* (“Hiei”). According to the Action Frame and Zhao teach transformation of cereals. The Action admits that neither Frame nor Zhao teaches selection at between about 30°C and about 34°C. However, the Action relies on Hiei to teach this element. Thus, the Action finds it would be *prima facie* obvious to combine the references to arrive at the invention. Applicants respectfully traverse.

The Action relied on column 7, lines 31-49 of Hiei for its alleged teachings. However, this relied upon portion relates to selection temperature for rice, not maize. Nowhere does Hiei teaches a selection temperature for maize. Rice and maize have different genetic architecture and physiological mechanism, and thus behave differently to environmental influences such that there would be no expectation of success with respect to maize. For example, rice genome size ranges between 415 and 460 Mb but maize genome size ranges between 2,300 and 2700 Mb. Rice is a C3 plant but maize is a C4 plant. Rice does not grow well in low temperatures, but maize grows well in relatively low temperatures. Accordingly, rice and maize respond differently. Therefore, a selection temperature for rice does not teach or suggest anything for determining an optimal selection temperature for maize.

The Action at page 3 states that “[g]iven the state of the prior art showing the differences of selection temperatures used for various explants, organisms...undue experimentation would be required to determine the effect of increased selection temperature on the transformation efficiency for any organism.” Thus, the Action acknowledges that a selection temperature for a given organism is unpredictable because extensive research is required to identify the conditions effective for each specific organism. This is because plant species are sufficiently diverse that each species may respond differently and exhibit different sensitivity to different temperatures.

Accordingly, one of skill in the art would not expect any success for maize selection temperature based on Hiei's teachings on rice.

The Action at page 3 also states that "the effect of increased temperature during selection on the transformation efficiency for various transformation methods using various explants are not well known in the art." Thus, Applicants note that the Action also acknowledges that the cited art is lacking any suggestion or motivation to select transformed cells at higher temperature range defined by the present claims. In fact, at the time of filing the current application, selection temperature was not regarded as an important parameter for investigation in studies of transformation efficiency. There is therefore no basis for the obviousness rejection. Nothing in the cited art suggests that benefit could be obtained by increasing selection temperatures or in particular by use of selection at 30°C or higher in maize. Selection temperature is not a parameter that would routinely be varied, absent the present disclosure. In contrast, Applicants have shown that such conditions may be used to achieve increased transformation frequency as seen in Example 8 and Table 3 of the specification. Accordingly, one of skill in the art would have been without reasonable expectation at the time of filing of the present application that increasing the selection temperature would increase transformation frequency.

Moreover, the increase in transformation frequency upon selection at higher temperatures is conceded to be unexpected. In particular, the previous Action states "[i]t may be true that the increase in transformation frequency upon selection at high temperature is unexpected...." *See* Action dated June 27, 2007, page 6. Applicants respectfully submit that the invention can not simultaneously be both obvious and unexpected.

In sum, neither Frame nor Zhao is asserted to teach the claimed selection temperature discussed above, and thus neither Frame nor Zhao cures the defects in Hiei. The rejection is therefore believed moot and removal thereof is respectfully requested.

**E. New Claim 31**

New claim 31 recites, “culturing the transformed maize tissue at a first temperature for a first period of time and at a second temperature for a second period of time...wherein the first temperature ranges from about 30°C to about 34°C and the second temperature is about 27°C.” Accordingly, new claim 31 requires a dual selection temperature regime for selection of transformed tissues. Example 8, Table 3 of the specification shows that the claimed dual selection temperature regime yielded transformation frequency ranging from 6.7% to 8.4%. As discussed above, the cited references do not teach selection temperature for maize, and thus, one of skill in the art would have been without reasonable expectation at the time of filing of the patent application that the claimed dual selection temperature would yield transformation frequency ranging from 6.7% to 8.4% in maize. Accordingly, the results were unpredictable and unexpected in light of the cited references. Therefore, allowance of claim 31 is respectfully requested.

**CONCLUSION**

In view of the foregoing, Applicants respectfully request favorable consideration of this case.

The Examiner is invited to contact the undersigned attorney at (214) 259-0931 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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